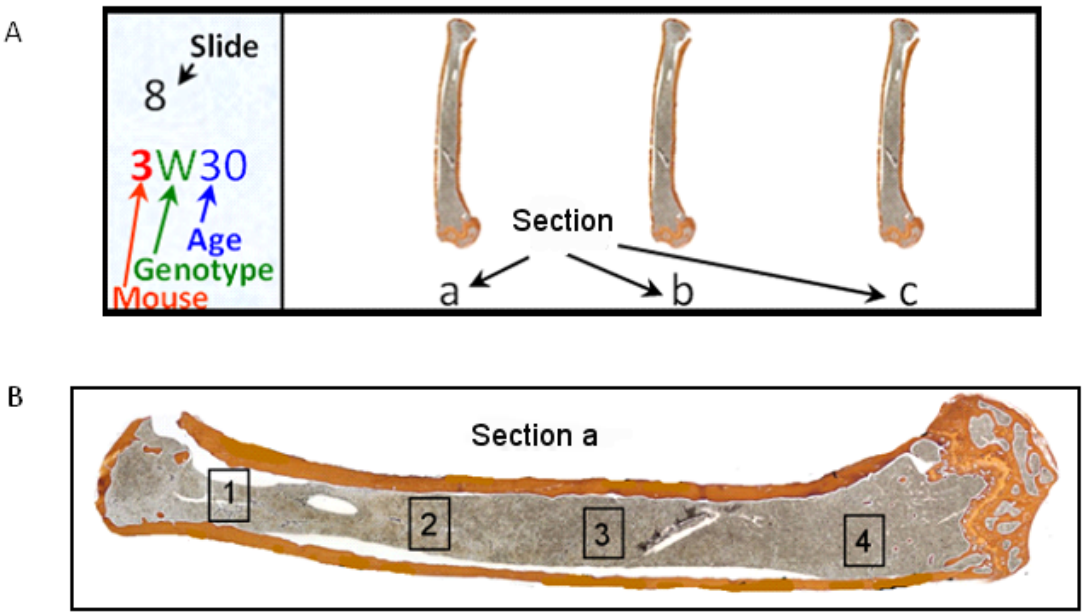
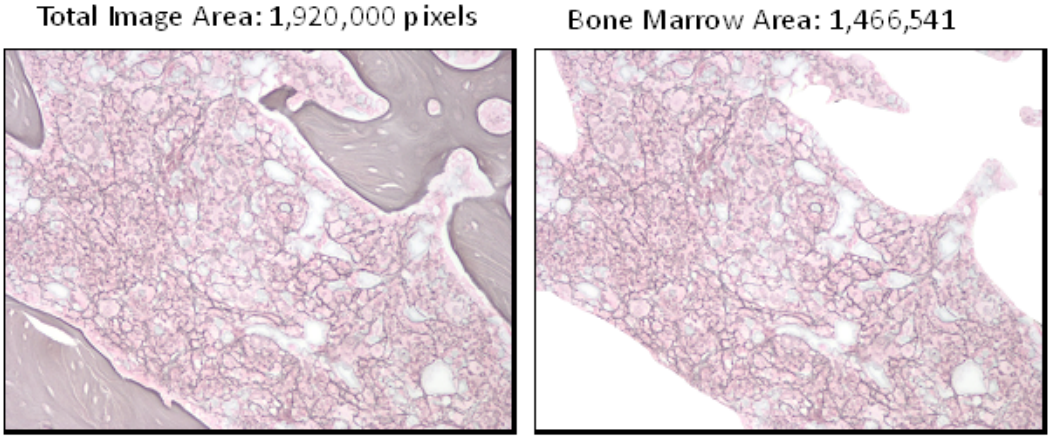


Supplementary Figure S1. Slides arrangement: A) Three bone sections corresponding to 5 µm thick, consecutive cuts are place in a slide. The slides labels include: slide number, animal ID, genotype and animal age. The bone sections are labeled "a", "b" and "c" from the slide head to the bottom. A unique ID of the pictures taken from bone slices is facilitated by this arrangement**. B)** Illustrates the partial imaging collected along the femur diaphysis longitudinal axis.

A

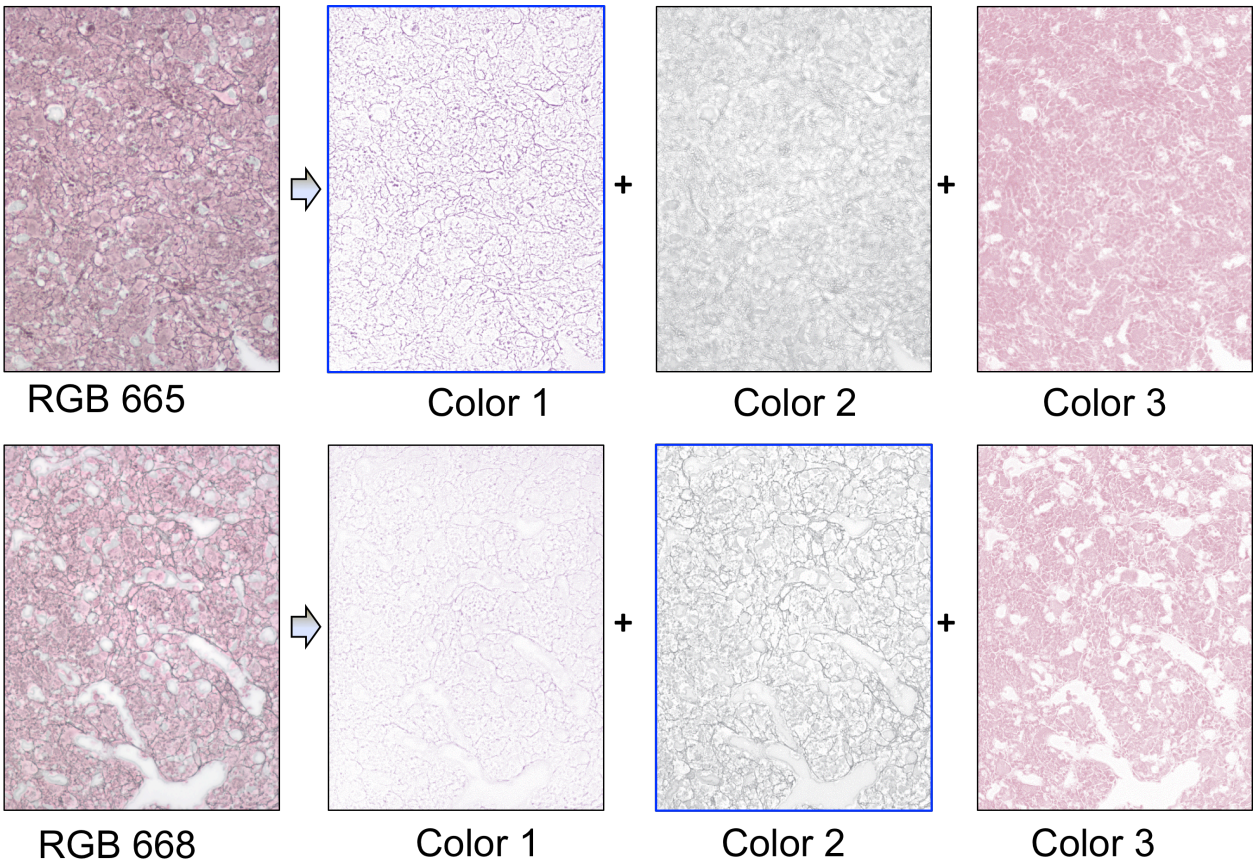


Ratio: Total Image Area/Bone Marrow Area =1.31

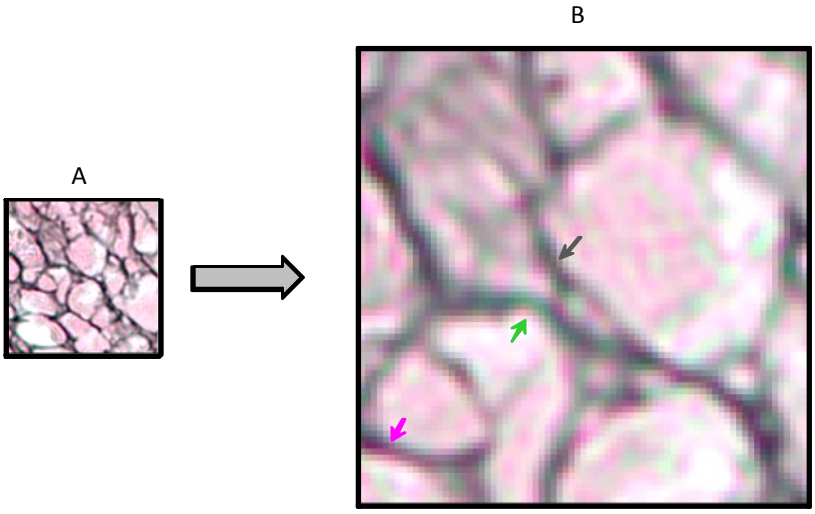
B

Area Correction Record					Normalization of IntDen	
Number	Image Name	TIA	BMA	TIA/BMA	Raw IntDen	Normalized IntDen
1	1G30_8_a.tif	1,920,000	1,920,000	**1.00**	35,728	**35,728**
2	1G30_8_a_2.tif	1,920,000	1,753,028	**1.10**	32,315	**35,393**
3	1G30_8_a_3.tif	1,920,000	1,580,653	**1.21**	29,650	**36,015**
4	1G30_8_a_4.tif	1,920,000	1,807,321	**1.06**	33,420	**35,504**
Key: TIA, Total Image Area; BMA, Bone Marrow Area.						

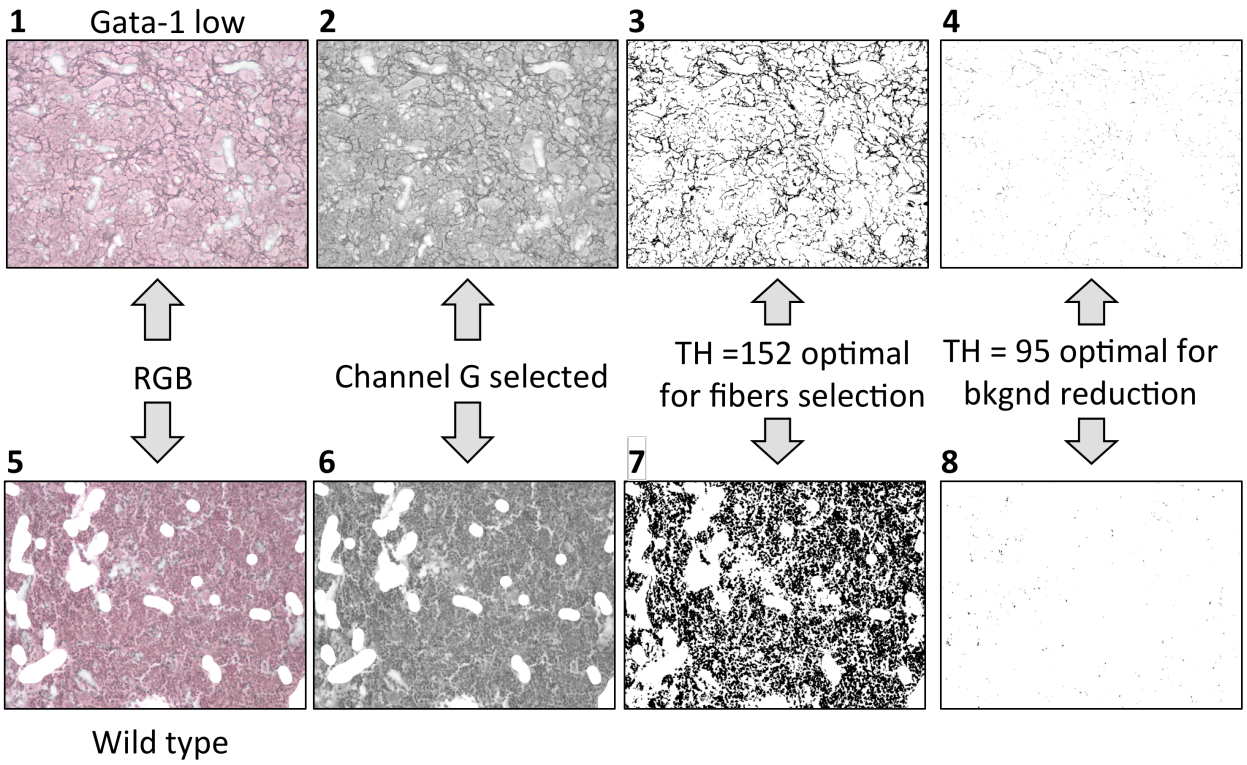
Supplementary Figure S2. A. Representative image correction of a 30 week old Gata-1low male mouse. Depicted on the left is the image with bone area and on the right the image after whitening the bone region. **B. Actual Bone Marrow Area.** Calculation of BM area in an image was described under Materials and Methods. This step also allows for the elimination of image artifacts and bone areas (image on the left). The table shows the output results and the normalization of raw IntDen by total image area. For a large number of images this process can be batched using the macro detailed in Supplementary Script S1.



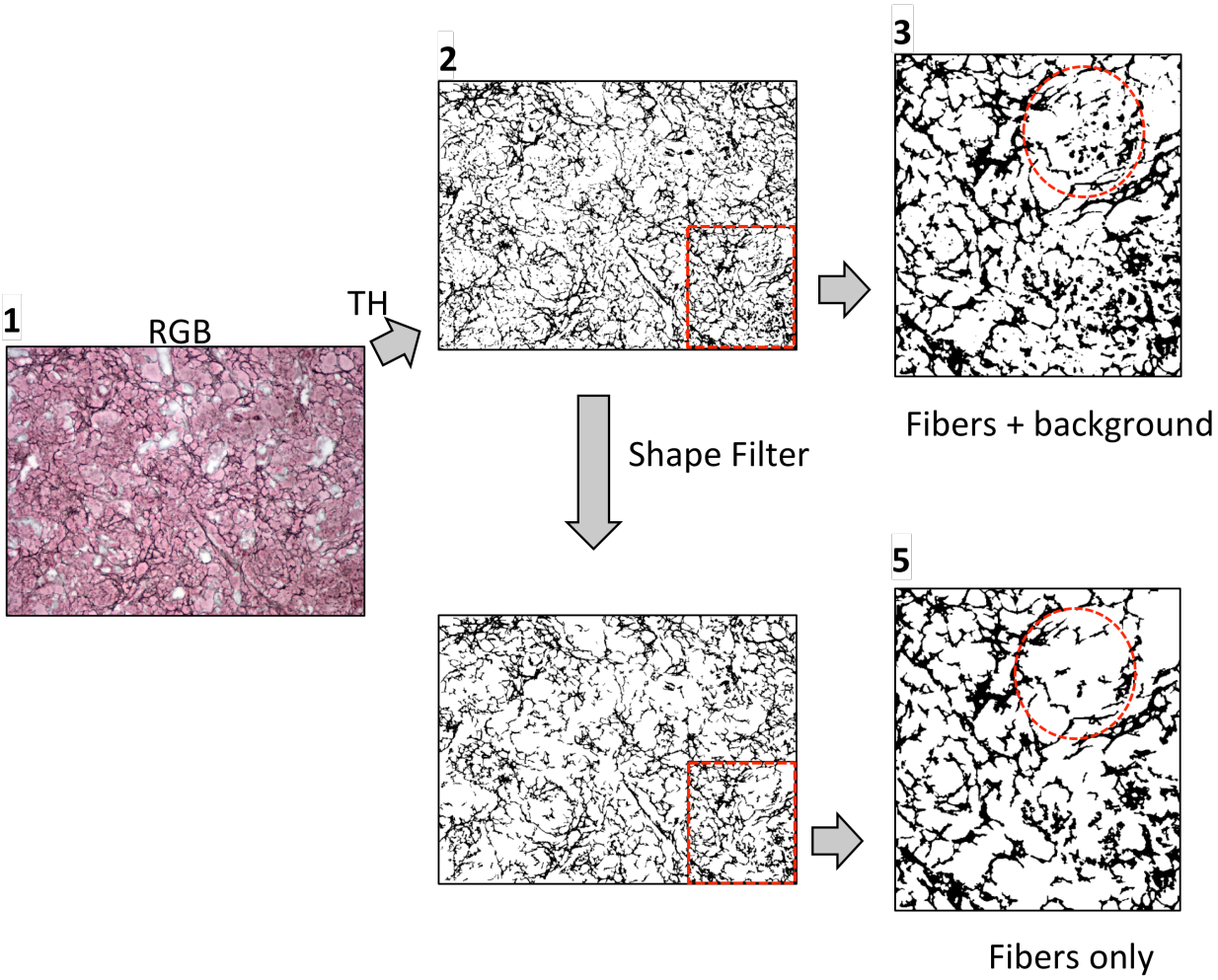
Supplementary Figure S3. Color Deconvolution of Gomori Silver Impregnated BM Reticulin Fibers. Two color images (665 and 668) from the same Gata-1 low male animal at 30 weeks of age were compared after color deconvolution (color1, color 2 and color 3 channels) using the Region Of Interest (ROI) values optimal for image 665. After color deconvolution, reticulin fibers from these images localized in different color channels (blue framed). User-defined ROI values for ImageJ color deconvolution analysis were: Color 1 = Red: 0.5015, Green: 0.7554, Blue: 0.4216; Color 2 = Red: 0.5917, Green: 0.5840, Blue: 0.5556, Color 3 = Red: 0.3191, Green: 0.7587, Blue: 0.5679.



Supplementary Figure S4. Polychromatic Nature of Reticulin Fibers Stained by Silver Impregnation: A region of Gomori stained reticulin (A) is magnified to the point where fibers show jagged pixel edges (B). This reveals the color heterogeneity of the stained fibers (arrows).



Supplementary Figure S5. Constant vs. Variable Thresholding. 20 weeks old male Gata-1 low (1) and matching male wild type (5) RGB images are split into their Red, Green and Blue channels and the G channels (2 and 6) are selected for threshold (TH) segmentation to isolate fiber shapes (3 and 7). A value of TH = 152 is optimal for Gata-1 low (3) while the same value is too high for the wild type (7). Conversely, a value of TH = 95 is optimal for wild type (8) but too low for Gata-1 low.



Supplementary Figure S6. Elimination of Background by Shape Filtering. The initial RGB image from 30 weeks old Gata-1low male mouse, used as representative analysis (1) is split into grey tone channels (not shown) and then thresholded to retain mostly fiber shapes (2). However, a high background of circular shapes, from stained nuclei, still remains (3, area circled in red). Filtering by shape to eliminate circular elements (4) eliminates the background (5, area circled in red)

Supplemental Script S1

Area Correction Macro (IJM extension)

```
 1 dir1 = getDirectory("C://Users//UserName//Desktop//In");
 2 dir2 = getDirectory("C://Users//UserName//Desktop//Out");
 3 setBatchMode(true);
 4 list = getFileList(dir1);
 5 for (i=0; i<list.length; i++) {
 6 showProgress(i+1, list.length);
 7 open(dir1+list[i]);
 8 setBatchMode(false);
 9 waitForUser("Delete bone area and spots, measure Bone Marrow
area");
10 run("Set Measurements...", "area display redirect=None
15decimal=0");
11 run("Measure");
12 saveAs("TIFF", dir2+list[i]);
13 close();
14 }
```

Supplemental Script S2

Manual Threshold, Shape-Filter Macro and Quantitation (IJM extension)

```
dir1 = getDirectory("C://Users//Hector//Desktop//In");
dir2 = getDirectory("C://Users//Hector//Desktop//Out");
setBatchMode(true);
list = getFileList(dir1);
for (i=0; i<list.length; i++) {
showProgress(i+1, list.length);
open(dir1+list[i]);
setBatchMode(false);
run("RGB Stack");
run("Arrange Channels...", "new=2");
run("Threshold...");
waitForUser("Set the threshold that will include only fiber structures");
run("Set Measurements...","integrated limit display redirect=None decimal=0");
run("Measure");
run("Convert to Mask", "method=Default background=Light");
saveAs("TIFF", dir2+list[i]);
close();
}
macro "2nd"{
dir1 = getDirectory("C://Users//Hector//Desktop//In");
dir2 = getDirectory("C://Users//Hector//Desktop//Out");
setBatchMode(true);
list = getFileList(dir1);
for (i=0; i<list.length; i++) {
showProgress(i+1, list.length);
open(dir1+list[i]);
setBatchMode(false);
run("Invert");
run("Shape Filter", "area=70-100000 area_convex_hull=0-Infinity perimeter=0-Infinity perimeter_convex_hull=0-Infinity feret_diameter=15-Infinity min._feret_diameter=0-Infinity long_side_min._bounding_rect.=0-Infinity short_side_min._bounding_rect.=0-Infinity aspect_ratio=1-Infinity area_to_perimeter_ratio=1.0-Infinity circularity=30-Infinity elongation=0-1 convexity=0-1 solidity=0-1 num._of_holes=0-Infinity thinnes_ratio=0-1 contour_temperatur=0-1 fractal_box_dimension=0-2 option->box-sizes=2,3,4,6,8,12,16,32,64 draw_holes slice");
run("Divide...", "value=255 slice");
run("Set Measurements...", "integrated limit display redirect=None decimal=0");
```

```
32 run("Measure");
33 saveAs("TIFF", dir2+list[i]);
34 close();
35 }
```

Supplementary Table S1. Correlation Between Image Original ID and Its Blind-coded ID

Coding from OI-id to #CI-id			Coding from #CI-id to 20DCI-id		Order of images for input analysis
List #	OI-id	#CI-id	#CI-id	20DCI-id	Order of 20DCI-id images in the folder for blind analysis
1	1G30_8_a.tif	1.tif	1.tif	A_OR66X_QU0UX_6FPC2_WZX5	0_44PB2_RB8WO_DVIYZ_774M.tif
2	1G30_8_a_2.tif	2.tif	10.tif	N_8CNIV_80EUP_NIMCL_0CWO	1_NCKCX_DCMV1_1DCI2_HN09.tif
3	1G30_8_a_3.tif	3.tif	11.tif	P_LII0L_YB4HC_9VVTA_N6QU	2_EO9JP_M2T7F_A767L_7TJX.tif
4	1G30_8_a_4.tif	4.tif	12.tif	B_I2D65_DOI6O_8AOTO_94I1	3_08AYB_MPEAH_HAOSD_VVF6.tif
5	1G30_8_a_5.tif	5.tif	13.tif	4_RO0QW_TDMIJ_6C7UK_HVI0	3_TZP8K_HJ559_PPPFN_8Q6H.tif
6	1G30_8_a_6.tif	6.tif	14.tif	9_D8XNL_SY1GZ_CWIJ1_OC6O	3_X6TVP_HXEAJ_724HX_6A8W.tif
7	1G30_8_b.tif	7.tif	15.tif	W_APL2J_ER0QV_ASM2Q_L6A5	4_2328S_4CKBV_QJ39Y_MAGJ.tif
8	1G30_8_b_2.tif	8.tif	16.tif	B_DT9BW_FVG6B_U9FVD_E422	4_LQKNZ_JO14C_GR2PF_5DXX.tif
9	1G30_8_b_3.tif	9.tif	17.tif	I_8I0U0_6Q910_X14E4_6X8I	4_RO0QW_TDMIJ_6C7UK_HVI0.tif
10	1G30_8_b_4.tif	10.tif	18.tif	B_EJTET_916LV_QWYTW_L66Q	7_6XCCG_U0VLU_WJ3W1_DRC0.tif
11	1G30_8_b_5.tif	11.tif	19.tif	R_1GKUG_6CE2T_4I4CP_JZT6	7_P97V4_L0VSU_FR6AV_K9KP.tif
12	1G30_8_b_6.tif	12.tif	2.tif	G_QM3LH_DU9J5_804G1_B3LF	8_60PGG_UBJ8P_D7VK4_XWED.tif
13	1G30_8_c_1.tif	13.tif	20.tif	C_437YY_9XI4U_MG33M_4848	9_D8XNL_SY1GZ_CWIJ1_OC6O.tif
14	1G30_8_c_2.tif	14.tif	21.tif	X_WBNRK_P2CCO_9JH1S_6VSJ	A_OR66X_QU0UX_6FPC2_WZX5.tif

The list of image files is organized in an order convenient for latter calculations. In this example only 14 out of 202 files are shown. The original image ID (OI-id) is first number coded (#CI-id) and then 20 digit coded (20DCI-id) for analysis. As a result, the 20DCI-id list order is also scrambled with respect to the OI-id list order. Finally, the far right column shows that the order of images for input analysis is further scrambled. The number coding step (#CI-id column) was obtained using the public domain Bulk Rename Utility (http://www.bulkrenameutility.co.uk/Main_Intro.php). Matching colored cells indicate order scrambling. The 20DCI-id was obtained using the BAR utility in ImageJ, as described under Bone Marrow Imaging in Methods. Key: **OI-id**, Original Image id; **#CI-id**, Number Coded Image id; **20DCI-id**, 20 Digits Coded Image id

Supplementary Table S2. Output data from Macro in Supplementary Script S2

Number	Image Name	IntDen	TH	Average	SD
1	1.tif	4,511,938	**109**	**109**	**2**
2	10.tif	3,064,318	**111**		
3	11.tif	3,326,377	**111**		
4	12.tif	2,856,948	**113**		
5	13.tif	3,252,806	**105**		
6	14.tif	5,690,022	**108**		
7	2.tif	6,126,860	**108**		
8	3.tif	5,273,364	**108**		
9	4.tif	3,886,046	**111**		
10	1.tif	**17,869**	255	**12,295**	**4,728**
11	10.tif	**4,431**	255		
12	11.tif	**9,491**	255		
13	12.tif	**7,609**	255		
14	13.tif	**10,912**	255		
15	14.tif	**17,639**	255		
16	2.tif	**19,863**	255		
17	3.tif	**14,150**	255		
18	4.tif	**8,547**	255		

Typical output results of the dual macro executed in the processing of 9 images. Note that the first sub-macro records the threshold (TH) input by the operator (rows 1-9, column TH). Values in column IntDen are irrelevant for this step. The second sub-macro generates data in rows 10-18, column IntDen. In this step TH column values are irrelevant. The average and standard deviations for TH and IntDen of the 9 images are shown in the far right columns